Optical clearing of human skin: comparative study of permeability and dehydration of intact and photothermally perforated skin

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Abstract. Accelerated diffusion of water and hyperosmotic optical clearing agents is studied as a result of enhanced epidermal permeability. A lattice of microzones (islets) of damage in stratum corneum is induced using a flash-lamp appliqué system. An optical clearing agent composed of 88% glycerol in aqueous solution is used for all experiments. Research of skin dehydration and glycerol delivery through epidermis at both intact and perforated stratum corneum is presented. The dehydration process induced by both stimuli of evaporation and osmotic agent action is studied by weight measurements. Dynamics of refractive index alteration of both glycerol solution and water during their interaction with skin samples is monitored. The amounts of water escaping from skin through the stratum corneum, due to hyperosmotic-agent action, and glycerol penetrating through the skin sample, are estimated. The results show that the proposed method allows for effective transepidermal water loss and delivery of optical clearing agents. © 2008 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2899149]

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1. Introduction

Interest in the use of optical methods in laser diagnostics, therapy, and surgery is continuously increasing. However, the main limitation of optical methods is a low penetration depth of light and complex character of light interaction with the superficial tissue layers, caused by tissue scattering properties defining spectral and angular characteristics of light propagated within a tissue. One of the methods aimed at the increase of light penetration depth and smaller distortion of laser beams is the use of hyperosmotic optical clearing agents (OCAs). The response of tissue to an OCA is a reduction in light scattering and corresponding increase in optical transparency and smaller distortion of light beams.

Numerous publications discuss the advantages of tissue optical clearing using OCAs and understanding the mechanisms of clearing. There are a few main mechanisms of light scattering reduction induced by an OCA: 1. dehydration of tissue constituents, 2. partial replacement of the interstitial fluid by the immersion substance, and 3. structural modification or dissociation of collagen. Both the first and second processes mostly cause matching of the refractive indices of the tissue scatterers (cell compartments, collagen, and elastin fibers) and the cytoplasm and/or interstitial fluid. Tissue dehydration and structural modification lead to tissue shrinkage, i.e., to the near-order spatial correlation of scatterers and, as a result, the increased constructive interference of the elementary scattered fields in the forward direction and destructive interference in the perpendicular direction of the incident light, that may significantly increase tissue transmittance even at some refractive index mismatch. For some tissues and for the nonoptimized pH of clearing agents, tissue swelling may take place that could be considered as a competitive process in providing tissue optical clearing.

It was shown that dehydration induced by osmotic stimuli such as OCAs appears to be a primary mechanism of optical clearing in collagenous and cellular tissues, whereas dehydration induces the intrinsic matching effect. The fluid space between fibrils and organelles is filled by water and suspended proteins. Water escaping from tissue having a cellular or fibrillar structure is a more rapid process than OCA entering into tissue interstitial space, due to the fact that OCA has greater viscosity (low diffusion coefficient) than water. As water is removed from the intrafibrillar or intracellular space, soluble components of interstitial fluid become more concentrated and refractive index increases. The resulting intrinsic refractive index matching between fibrils or organelles and
their surrounding media, as well as density of packing and particle ordering, may significantly contribute to optical clearing. Replacement of water in the interstitial space by the immersion substance leads to additional matching of the refractive indices between tissue scatterers and ground matter.

OCAs used frequently are glycerol, glucose, mannitol, propylene glycol, polypropylene glycol, polyethylene glycol, x-ray contrasting agents (verografin, trazograph, and hypaque), 1,3-butanol, 1,4-butanediol, and their combinations. As enhancers of OCA diffusivity through stratum corneum, ethanol, dimethyl sulfoxide (DMSO), and linoleic and oleic acids are used. Sometimes DMSO alone is used as the OCA due to its own extremely high permeability (as a polar aprotic solvent of stratum corneum lipids) and its high index of refraction.

However, the optical clearing process in collagen-based tissues may involve a change in the supramolecular structure. Collagen reversible solubility in sugars and sugar alcohols may take place. It was demonstrated that glycerol treatment induces swelling of intercellular space and dissociation of collagen fibrils into microfibrils (loss of characteristic banding in some areas). Agent-induced destabilization of collagen structures may lead to additional reduction of optical scattering in tissue.

A number of laser surgery, therapy, and noninvasive diagnostic technologies may have a significant benefit from a reversible skin scattering reduction. However, slow diffusion of OCAs through a human skin barrier makes a practical application of optical immersion effect difficult. To reduce the barrier function of skin epidermis, a number of different chemical10,11 and physical methods such as stripping, microdermabrasion, laser ablation of skin surface, iontophoresis, ultrasound, and photomechanical waves12 were proposed. Physical methods for transdermal agent delivery have two advantages over formulations and chemical enhancers: 1) interaction between enhancer and the agents being delivered is absent; and 2) they reduce the risk of additional skin irritation. However, some methods, such as stripping and laser ablation of skin surface, can be related with the damage or ablation of large areas of stratum corneum. Iontophoresis results in a reduction of the resistance of the skin to diffusion of ions, but as an enhancer of the flux of uncharged molecules, it is a less effective method. Ultrasound and other pressure waves can affect various skin structures and inner organs due to a large penetration depth that can be undesirable for specific tasks of OCA delivery. There is therefore a need to develop alternative methods providing skin barrier permeability for OCAs.

Recently, a method of accelerating penetration of the OCAs due to enhancing epidermal permeability by creating a lattice of microzones (islets) of limited photothermal damage or lattice of islets of light perforation (LID) in the stratum corneum (SC) was proposed. LID are created as a result of absorption of a sufficient amount of optical energy by the lattice of microzones. The absorption leads to temperature elevation in the localized zones of interaction. Since the zones of interaction (microdots) contact the skin surface, some of the thermal energy will be transferred to the SC. As the damage of the SC is not damage of viable tissue, the long-term effect of such damage is only the transient deterioration of skin barrier function. That leads to the local increase of OCA penetration. The lattice of optical islets can be formed using a variety of energy sources and delivery optics, including application of lenslet arrays, phase masks, and matrices of exogenous chromophores.

The goal of this work is the investigation of the effectiveness of the LID method for enhancement of SC permeability for both water and OCAs. We present the experimental results on dehydration of ex-vivo human skin by the following stimuli: evaporation and application of OCA, and refractive index alteration dynamics of both water and OCA during their interaction with skin samples.

## 2 Materials and Methods

### 2.1 Tissue Samples

12 tissue samples of human skin from axillary space were included in the study. Eight of them were used in the dehydration study and four samples were used in refractometry experiments. Full-thickness samples were obtained postsurgically from middle-aged humans. The samples were stored in saline 20 to 24 h after excision. The area of the samples was about 20×30 mm. The samples were used after adipose layer removing. Thickness of the samples was measured by micrometer before dehydration and after. Accuracy of the measurements was ±50 μm. The values of thickness are presented in Table 1.

### 2.2 Immersion Agents

An optical clearing agent composed of 88% glycerol in aqueous solution was used. Refractive index of the glycerol solution measured by an Abbe refractometer was $n_\text{gl}=1.454$.

### 2.3 Flash-Lamp Appliqué System

For half of the skin samples, SC was perforated. For the perforation, a light/appliqué system including a flash lamp (EsteLux System, Palomar Medical Products Incorporated, Burlington, Massachusetts) and specially designed appliqué was used (see Fig. 1). The appliqué was a transparent plastic film with a pattern of absorbing centers (dots). Diameter of the dots was ~150 μm; distance between the dots was ~500 μm. Regime of the irradiation was two pulses with fluence 27 J/cm². The dots were made up of inert and biocompatible carbon powder, ensuring high absorption of light

<table>
<thead>
<tr>
<th>Dehydration stimuli</th>
<th>Before dehydration</th>
<th>After dehydration</th>
<th>SC perforation</th>
<th>$r$, hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>1.56±0.06</td>
<td>1.36±0.05</td>
<td>+</td>
<td>36.4±0.7</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.44±0.06</td>
<td>1.21±0.05</td>
<td>–</td>
<td>193.4±12.3</td>
</tr>
<tr>
<td>Evaporation</td>
<td>1.43±0.1</td>
<td>0.81±0.05</td>
<td>+</td>
<td>71.2±1.9</td>
</tr>
<tr>
<td>Evaporation</td>
<td>1.98±0.12</td>
<td>0.93±0.05</td>
<td>–</td>
<td>1408.9±36.3</td>
</tr>
</tbody>
</table>
energy. The dots absorbed the light energy, which resulted in rapid temperature elevation. The appliqué closely contacted the skin surface to avoid energy dissipation and localize light absorption, and thus the thermal damage, within the thin surface layer.

Parameters of the light/appliqué system were selected in such a way that no irreversible damage was caused to the SC, so that integrity of the skin barrier was restored in a short time. Such a regime of irradiation was earlier tested in an in vivo study.21 The size of the island damage mask (IDM) was 10 × 20 mm, corresponding to a handpiece window of the flash-lamp system. As a result of thermal action, LID with diameters of craters 200 ± 20 μm and depth 25 ± 5 μm arose on the surface of the skin samples. Sizes of the craters were estimated by use of a tape-stripping method.21 Some tape strips were removed from the skin treated by the flash-lamp appliqué system using film (Multi-Film, TESA, Beiersdorf, Hamburg). Removed tape strips were investigated using an optical microscope. Diameters of the holes in the layer of SC on the tape strips corresponded to diameters of the craters, and the thickness of the strips allowed estimating the depth of the craters in SC.

To remove products of the thermal action from the craters, an alcohol compress was applied on the skin surface for 15 min. Concentration of ethanol in the solution was 40%. This procedure also allowed us to enhance permeability of viable epidermis, especially within the zones in the projection of holes in the SC.

2.4 Estimation of Tissue Dehydration

Eight samples of skin with both perforated and intact SC were desiccated by either osmotic stimulus or evaporation. Figure 1 shows the design of the prepared sample. Sample 2 was placed on separate glass object-plates (1 in Fig. 2, dermis surface down) and covered by a polyethylene film (5 in Fig. 2) with a window 10 × 20 mm (3 in Fig. 2, corresponding to the damaged area 4) to exclude drying of the samples from the side of dermis. The surface of four samples was covered by the osmotic agent only in the area of the window. Adjacent tissue specimens prepared in the same way were dehydrated in air. All samples were dried at 20°C in a tightly closed box with silicone powder to prevent uncontrolled absorption of water from the environment by the samples.

The samples were weighed on an electronic balance every hour (excluding night) for a few weeks until a constant weight was reached by each sample. For the samples, which were desiccated by osmotic stimulus, the agent was removed thoroughly before weighing and then applied again after weighing. Experiments were performed at room temperature.

Tissue dehydration is related to diffusion of water from the tissue to an external volume that experimentally measured dynamics of the change of dehydration degree can be described on the basis of the equation of diffusion through a permeable membrane. For the estimation of tissue dehydration degree under the action of both air and a hyperosmotic agent, the equation can be used by analogy of the diffusion equation, which describes the flow of substance from a small volume with nonzero concentration into a reservoir with zero concentration:

\[
\frac{C(t)}{C_0} = \frac{M(t) - M_0}{M(t=0) - M_0} = \exp\left(-\frac{t}{\tau_D}\right),
\]

where \( M \) is a mass of the skin sample (g), \( M_0 \) is an additional term, which corresponds to the permanent mass of the sample (i.e., dry mass) (g), \( C \) is a concentration of the diffusing substance (g/ml), \( C_0 \) is a concentration of the substance in closed volume in initial time (g/ml), \( \tau_D \) is a time constant that characterizes the rate of dehydration (s), and \( t \) is time of dehydration (s). The parameter \( M_0 \) includes mass of collagen, elastin, and other components of tissue after its total dehydration, bound water, and possibility of residual free water. The difference between initial and permanent mass of the sample \([M(t=0) - M_0]\) shows that an amount of water escaped from the tissue due to dehydration.

However, both parameters \( M \) and \( M_0 \) depend on the initial mass of tissue sample. To exclude the dependence, we can write:

\[
H_D(t) = \frac{M(t) - M(t=0)}{M(t=0)} = \frac{M_{H_2O}(t)}{M(t=0)} = \frac{V_{H_2O}(t) \times \rho_{H_2O}}{M(t=0)},
\]

where \( H_D \) is a degree of tissue dehydration; \( M_{H_2O}(t) \) and \( V_{H_2O}(t) \) are mass (g) and the volume (ml) of water, respectively, the latter escapes from tissue in each moment during
dehydration; and $H_2O = 1 g/ml$ is water density. Substituting Eq. (1) into Eq. (2) and simplifying them, we obtain the following expression:

$$H_D(t) = \frac{M(t = 0) - M_0}{M(t = 0)} \left[ 1 - \exp(-t / \tau_D) \right], \quad (3)$$

which can be written in the form:

$$H_D(t) = A_D \left[ 1 - \exp(-t / \tau_D) \right]. \quad (4)$$

The parameters $A_D$ and $\tau_D$ were obtained by minimization of the target function:

$$F(A_D, \tau_D) = \sum_{i=1}^{N_i} \left[ H_D(A_i, \tau_i) - H_D^*(\tau_i) \right]^2, \quad (5)$$

where $H_D(A_i, \tau_i)$ and $H_D^*(\tau_i)$ are the calculated and experimental values of the time-dependent degree of dehydration, respectively; and $N_i$ is the number of time points obtained at registration of the temporal dynamics of the dehydration. To minimize the target function, the Levenberg-Marquardt nonlinear least-squares-fitting algorithm described in detail by Press et al. has been used. The iteration procedure repeats until experimental and calculated data are matched. As a termination condition of the iteration process, we have used the following expression:

$$\frac{1}{N_t} \sum_{i=1}^{N_t} \left| H_D(A_i, \tau_i) - H_D^*(\tau_i) \right| / H_D^*(\tau_i) \leq 0.01.$$

### 2.5 Refraction Index Measurements

A series of experiments on measurement of refractive index dynamics of OCA during its interaction with skin samples was carried out. Four samples were put into a temperature-controlled Franz cuvette (see Fig. 3). It included two reservoirs, 1 and 2, which were separated by a skin layer 4. Borders of the reservoirs were rigid and fixed tissue sample to exclude infiltration of the studied agent. For measurements of OCA refractive index dynamics, the upper reservoir 1 was filled up by 2 ml of the agent 3. The solution interacted with epidermis, which was perforated 5 or nonperforated by the flash-lamp appliqué system. The lower reservoir 2 was filled up by 20 ml of saline 6. It contacted with dermis and maintained the normal hydrated state of a skin sample. The low reservoir was attached to the thermostat. The temperature of saline was kept constant at about 38.5°C. The temperature of the skin surface in this case was about 34°C, which corresponds to the normal temperature of human skin surface.

For measurement of the change of water refractive index, the upper reservoir 1, on the contrary, was filled up by 2 ml of water, and the lower reservoir 2 was filled up by 20 ml of glycerol. Also, the skin sample was placed epidermis down, to make contact with the glycerol solution.

From the upper reservoir, 0.1 ml of the studied agent was taken one by one every half hour for measurement of the refractive index with an Abbe refractometer. Before taking the studied solution, it was mixed thoroughly directly in the reservoir. Time of interaction of tissue samples with the studied agents was 4 to 6 h.

### 3 Results and Discussion

Skin dehydration dynamics are demonstrated in Fig. 4. The experiment provided the skin dehydration by the following stimuli: evaporation (no application of OCA) (1 in Fig. 4) or application of OCA (2 in Fig. 4). The SC of the samples was perforated by the flash-lamp appliqué system. The symbols present the experimental data, and solid lines correspond to the result of modeling using Eq. 4. The vertical lines indicate the standard deviation values. Equation 4 allows us to estimate characteristic time $\tau_D$, showing the typical time of the water loss process. The values of the dehydration characteristic time for all investigated samples are presented in Table 1.

In Fig. 4 it is seen that tissue dehydration increases with time for all dehydration stimuli. At that the degree of dehydration by air is highest. The rate of tissue dehydration can be estimated from both parameters: characteristic time and the degree of dehydration. The estimation shows that dehydration rates of skin on air (1 in Fig. 4) and under glycerol action (2 in Fig. 4) are approximately equal.

The study of the evaporative process allows us to separate, in some sense, the dehydration mechanism of optical clearing.
Dehydration dynamics of human skin samples in air: 1 is the damaged stratum corneum and 2 is the intact stratum corneum. Symbols represent experimental data, solid lines correspond to approximated dependence, and the vertical lines are the standard deviation values.

Fig. 6 Dynamics of glycerol-induced dehydration of human skin samples with: 1 the damaged stratum corneum and 2 the intact stratum corneum. Symbols represent experimental data, solid lines correspond to approximated dependence, and the vertical lines are the standard deviation values.

Dehydration dynamics of the skin samples with both perforated (line 1) and intact (line 2) SC that were dehydrated by air. Symbols present the experimental data, solid lines correspond to approximated dependence, and the vertical lines are the standard deviation values. Figure 5 demonstrates that the degree of dehydration of both samples was practically equal, since they were dried in air in equal terms. However, from the analysis of the dehydration characteristic time of the samples (see Table 1), it is seen that dehydration of the first sample was more than 20-fold faster than the second one.

Dehydration dynamics of the human skin samples with and without LID under action of glycerol solution is demonstrated in Fig. 6. SC of the first sample was perforated, and that of the second one was intact. Symbols present the experimental data, solid lines correspond to approximated dependence, and the vertical lines are the standard deviation values. Table 1 shows that the characteristic time of dehydration for the first sample was shorter than for the second. However, the degree of dehydration of the sample with predamaged SC was lower than that of the intact sample.

Fig. 7 shows refractive index dynamics of glycerol during skin dehydration through epidermis for samples with both intact (line 1) and damaged (line 2) SC. For four hours, the refractive index of glycerol solution decreased insignificantly in the first case. But in the second case, the glycerol refractive index changed faster.

Based on the Gladstone-Dale law, which states that the resulting value of refractive index of solution represents an average of the refractive indices of the components related to their volume fractions, and refractive index measurements, it is possible to obtain the volume fraction of water escaping from the skin to the glycerol solution through epidermis:

\[
    n_{\text{solution}} = n_{\text{water}}C_{\text{water}} + n_{\text{gl}}C_{\text{gl}},
\]

where \( n_{\text{solution}} \), \( n_{\text{water}} \), and \( n_{\text{gl}} \) are refractive indices of environmental solution in which refractive index was measured, water and glycerol, respectively, and \( C_{\text{water}} \) and \( C_{\text{gl}} \) are volume fractions of water and glycerol in environmental solution, respectively.

Taking into account that the immersion agent volume changes during the measurements, we estimated water volume in the glycerol solution. Figure 7 shows the change of water volume in glycerol solution due to water diffusion from skin through both nonperforated (line 1) and perforated (line 2) SC. It is seen that for the sample with perforated SC, the volume of additional water in the glycerol solution increased from zero to 0.05 ml in four hours. It is more than 4% of the total volume of glycerol solution. For the sample with intact SC, the increase of water content was not significant (about 1%).

The study of dynamics of refractive indices of water at the interaction of skin with an osmotic agent was carried out. In this case, skin dermis in contact with water and epidermis with or without LID was washed by glycerol solution. The thickness of the samples selected for the experiment was less than the others, only 0.8 ± 0.2 mm, because we wanted to detect glycerol by the change of the refractive index in water from opposite sides of the sample. Due to the thin samples,
we could register the penetration of glycerol through skin without long-term storage, when uncontrolled change of both the physical and biochemical properties of a sample could take place. Results of the measurements are presented in Fig.

Penetration of the glycerol solution through the skin is accelerated by creating the corresponding pattern of enhanced permeability channels. Figure shows the dynamics of glycerol amount in water due to diffusion through skin estimated by the Gladstone-Dale law

The increase of glycerol volume in water was about 3.2 and 4.5% (from the total volume of water) at its penetration through skin with intact and perforated SC, respectively.

The change of mass of the skin samples exposed to both air and OCA is closely related to water loss, because water is the most mobile component of skin. In the case of air stimuli, water evaporates from tissue, and in the case of osmotic stimuli, water escape takes place due to osmosis. Therefore, dehydration of skin can be estimated from weight measurements. However, under the influence of air stimuli, dehydration of tissue manifested in the decrease of mass of a sample is related only to water loss. Under action of OCA, the final mass of a sample includes dry matter, residual mass of water, and the agent penetrated into the tissue.

It is known that the use of an osmotic agent, such as glycerol, promotes acceleration of skin dehydration. Results have shown that equal dehydration of skin through nondamaged SC, for example 45%, can be provided by glycerol in about 300 h and by air in about 600 h (see sample 2 in Figs. and

However, it is seen that the degree of resulting dehydration under influence of the osmotic agents is lower than that of air. This effect could be related to glycerol penetration into the skin, even through intact SC. [Refractive index measurements confirm the fact (see Fig. 9). We can see that the refractive index of water increases not only for the sample with LID in SC of epidermis, but for the sample without it. This could be explained by penetration of glycerol through tissue in both cases].

On the one hand, glycerol, as an osmotic active agent, stimulates diffusion flow of free water from tissue to OCAs solution. On the other hand, it prevents total dehydration of tissue due to holding water inside, as water dilutes an initial glycerol solution. In contrast, for skin dehydration in air, free water evaporates from tissue almost completely.

Damaging of SC provides significant acceleration of the dehydration process from osmotic stimuli as well as in air, since formed craters serve as centers of enhanced permeability of epidermis, not only to water escaping from skin but to hydrophilic agents, like glycerol (see sample 1 in Figs. and

Comparison of the rates of dehydration of skin by both stimuli of air and glycerol at damaged SC has shown that the rates of both processes are equal (see Fig. 4). It may be supposed that in the initial time, only dehydration of skin takes place for both stimuli. Gradually, glycerol solution replaces water in interstitial liquid and the dehydration process stops (see sample 2 in Fig. 4), whereas for air stimuli, water loss continues to total drying of the sample (see sample 1 in Fig. 4).

For the analysis of the dehydration process induced by glycerol (see Fig. 6), it can be seen that the degree of dehydration decreases for the samples with predamaged SC. As noted before, in both cases two flows take place: the first one...
is water diffusion from the low layer of epidermis and from dermis to the applied agent, and the second one is agent diffusion from an environment solution into tissue. In the non-perforated sample, apparently, the second flow is less than the perforated one; therefore, as a result the degree of dehydration of the sample without LID is larger than that of the sample with LID. Thus, free admission of the agent into skin prevents formation of high concentration gradient between interstitial water and environment solution that decreases total dehydration of tissue.

Results presented in Figs. confirm that two processes—water loss and osmotic agent diffusion into skin—took place simultaneously. The results of the study of refractive index dynamics demonstrate that the diffusion rate of water from skin through the epidermis to an environment solution, as well as glycerol through skin, increase with the use of LID. In the initial stage (a few hours), which is most interesting for medical applications, LID promotes better penetration of immersion agents into tissue, which induces a higher clearing degree in a shorter period. Results of spectral measurements performed in [32] have shown that the reflectance of human skin in vitro for the case of predamaged SC decreased practically two-fold in an hour, which is unachievable for the topical application of OCA on the intact skin surface.

The perforation of SC apparently influences not only the rate but the mechanism of the clearing process. In the case of interaction of glycerol solution with intact skin prevalent, the mechanism of clearing may be considered as dehydration because the SC barrier prevents free diffusion of OCA into skin. For damaged SC, the dehydration of tissue accelerates, but the refractive index matching effect also has a great importance as the OCA solution penetrates immediately in the interstitial space. In experiments presented in Ref. [32] the LID method was tested on human skin in vivo. To prove that formed damage islets serve as centers of enhanced permeability of epidermis to hydrophilic agents, glycerol solution with methylene blue added was applied to the treated skin area with LID. Damaged areas were stained more intensively than intact skin. Also, spread of the dye under the skin surface was observed. However, quantitative estimation of both degree dehydration and clearing of human skin in vivo was not performed. The dehydration process of skin in vivo can be partly compensated in many respects by water diffusing from environment tissue to the place of OCA action due to the metabolism of living tissue.

On the basis of our results, we can assume that the dehydration effect is expected to be less significant.

4 Conclusion

Results of the experiments show that the LID method is effective for transepidermal delivery of optical clearing agents. Microdamage of the skin surface serves to reduce the stratum corneum barrier function and promote both enhancement of water escape from skin and penetration of a hyperosmotic agent into skin. Free admission of clearing agents into skin prevents the formation of high concentration gradients between interstitial water and environment solution that decreases the dehydration of tissue and can be considered a positive factor for in vivo skin optical clearing, since dehydration relates to decreasing the rate of the exchange process.

Significant change of refractive indices of osmotic solutions contacted with damaged stratum corneum in comparison with intact ones is the evidence of additional water loss at its perforation. Also, we succeed in the estimation of amounts of water escaping from skin and glycerol transport through skin 4 to 6 h after interaction of glycerol with the tissue surface.

The island damage method could be used successfully in in vivo conditions for the enhancement of optical clearing of the treated skin area and as a faster effect for medication.

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